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THE INFLUENCE OF TEMPERATURE UPON THE COMBINATION OF OXYGEN WITH THE BLOOD OF TROUT [1,2]

LAURENCE IRVING, EDGAR C. BLACK AND VIRGINIA SAFFORD

(From the Edward Martin Biological Laboratory, Swarthmore College, Swarthmore, Pennsylvania)

The air breathed by all species of mammals is the same in composition, and the affinity of their blood for oxygen is also much alike. But the water in which fish live differs greatly in oxygen saturation in different places and seasons, and this variability is particularly conspicuous in bodies of fresh water in temperate regions. Under these circumstances it is not surprising to find that the blood of various species of fish varies greatly in affinity for oxygen. Krogh and Leitch (1919) first remarked upon the differences among the eel, carp, plaice, cod, and trout, and regarded these differences in affinity for oxygen as evidence for the adaptation of the blood to the conditions in which each species lived. But only a few species were examined and these were quite dissimilar in form and habit as well as in respect to the habitat which they occupied.

The catfish, carp, bowfin, and sucker were found by Black (1940) to have blood with different oxygen dissociation curves, which were nevertheless related as if in one family. These freshwater fish are similar in form and are all of free swimming habit. Since their respiratory requirements and their physical systems for providing oxygen are much alike, it is reasonable to consider that large differences in the properties of the blood of these species indicate the suitability of the blood for respiration in different environments.

We have now examined the blood of eight species of freshwater fish and find that the affinity for oxygen varies greatly among the species. Furthermore, as Black (1940) pointed out for four of the species, the

effect of CO_2 upon oxygen combination is also quite different in the species. Table I shows the usual oxygen affinity and the effect of CO_2 in the blood of these eight species. These characteristics would fit respiration under quite different conditions.

These properties of the blood do not depict its natural suitability for the transport of oxygen because there is no allowance for the variety

TABLE I

Oxygen affinity and effect of CO_2 in blood of freshwater fish at 15°
(Jordan, 1929)

Species	$P CO_2$ at $\frac{1}{2}$ saturation		Limit of CO_2 effect
	$P CO_2 = 1-2$ mm.	$P CO_2 = 10$ mm.	Percentage HbO_2
Common catfish *Ameiurus nebulosus*	1.4	5	(Black, 1940)
Bowfin *Amia calva*	4	9	(Black, 1940)
Carp *Carpiodes cyprinus*	5	8	85 (Black, 1940)
Yellow perch *Perca flavescens*			77 (Irving, unpublished)
Common sucker *Catostomus commersonnii*	12	43	71 (Black, 1940)
Chain pickerel *Esox niger*			53 (Irving, unpublished)
Rainbow trout *Trutta iridea*	18	35	52 (Irving, Black, and Safford— this paper)
Brown trout *Trutta trutta*	17	39	52 (Irving, Black, and Safford— this paper)
Brook trout *Salvelinus fontinalis*	17	42	52 (Irving, Black, and Safford— this paper)
Atlantic salmon (freshwater) *Salmo salar*	19	35	57 (Irving, 1939)
Lake trout *Cristivomer namaycush*			40 (Irving, unpublished)

of temperatures in the natural habitats of the fish. In lakes in temperate regions the temperature of a stratum of water may differ sharply from the temperature above and below, and the seasonal changes are rapid and large. The influence of temperature upon the oxygenation of mammalian blood is such that at 20° it would be 95 per cent saturated with oxygen by a pressure of 45 mm., while at 37° the pressure required is about 100 mm. (Brown and Hill, 1923). According to the figures

given for the blood of the skate (Dill et al, 1932), the hemoglobin would be 95 per cent saturated by 40 mm. pressure of oxygen at 15°, but the same saturation would require 170 mm. at 25°. At the higher temperature part of the efficacy of the hemoglobin for oxygen transport would be lost even in water which was saturated with air. The blood of the eel would be 95 per cent saturated with oxygen by pressures of 12, 25, and 71 mm. at 5°, 17°, and 30° respectively (Kawamoto, 1929). From general considerations concerning the nature of hemoglobin, as well as from these two examples, it is to be expected that the function of oxygen transport in fish blood is considerably influenced by the temperature at which it occurs.

The combination of oxygen with the blood of fish is peculiarly sensitive to carbon dioxide, as Krogh and Leitch (1919) first observed. Even at 700 mm. pressure of oxygen the hemoglobin of the blood of the tautog will not become saturated with oxygen in the presence of carbon dioxide (Root and Irving, 1940). In this respect the CO_2 effect in fish blood differs from the Bohr effect produced by CO_2 in mammalian blood. In the practical absence of CO_2 the hemoglobin of fish blood is saturated with oxygen at 150 mm. pressure. With increasing pressures of CO_2, oxygen saturation falls off until no further reduction of oxygen saturation is secured beyond 60 mm. pressure of CO_2 (Root, Irving, and Black, 1939). At the limit of the CO_2 effect on the blood of trout only about 50 per cent of the hemoglobin can be oxygenated.

The limit of the CO_2 effect is quite different in various species of fish. A number of freshwater fish which we have examined clearly show the variation in the limit of the CO_2 effect in the blood of several species (Table I, column 3). The differences in CO_2 sensitivity of the blood and the influence which the CO_2 effect would exert upon conditions essential for respiratory transport, however, have only been determined at 15°.

The limit of the CO_2 effect in trout blood changes with temperature, as is shown by the observations recorded in Fig. 1. These determinations were made by measuring the percentage of saturation of the hemoglobin with oxygen at a pressure of 150 mm. and CO_2 at 65 mm. or more. The blood samples were secured from a number of brook, brown, and rainbow trout. The limit of the CO_2 effect falls at a lower level of oxygen saturation with increasing temperature up to 25°. Above 25° the blood cannot be saturated by oxygen at 150 mm. pressure even in the absence of CO_2.

The influence which temperature exerts upon the CO_2 effect shows that the effect of CO_2 upon affinity for O_2 must be considered in making

a description of the blood of fish which will be useful in designating its suitability for the performance of its natural function of oxygen transport.

MATERIAL USED

For examining the effect of temperature upon oxygen affinity and the CO_2 effect we have selected three common and related species of freshwater fish; brook trout, *Salvelinus fontinalis;* brown trout, *Trutta*
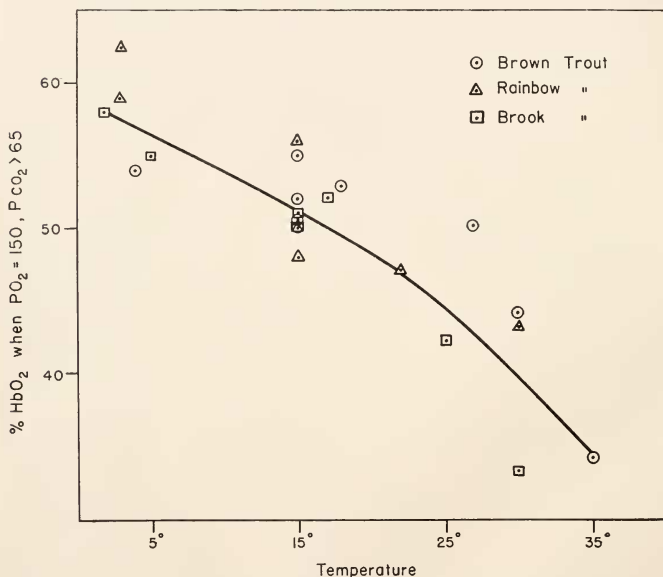

FIG. 1. Limit of the effect of CO_2 upon oxygenation of hemoglobin in trout blood.

trutta; and rainbow trout, *Trutta iridea.* These fish live under similar conditions and are alike in activity. They were raised in the same water at the New Jersey State Hatchery at Hackettstown. All had received the same sort of food, and each species had been raised from a homogeneous and selected stock. If they were as different in form and habits as the toadfish and the tautog, all of the physical components of the respiratory mechanism would obviously be different, and a comparison of the species on the basis of the properties of blood alone would

not be justified. But since these three species of trout are so much alike, against the background of general similarity of essential respiratory devices either similarity or difference in the properties of the blood for respiratory transport may acquire significance for the eventual picture of specific respiratory adaptation.

METHODS

Male fish of from $1\frac{1}{2}$ to $2\frac{1}{2}$ pounds weight were bled by syringe from the heart, yielding from 5 to 20 ml. per fish. Coagulation was prevented by liquid heparin (Connaught Laboratory), the blood was iced, and analysis was begun about five hours after drawing and completed within the next fourteen hours. About five of the eighty fish used died but the others showed no ill effects from bleeding.

The blood was equilibrated for more than 20 minutes with suitable gas mixtures. Half-milliliter samples were withdrawn and analyzed according to the method of Van Slyke and Neill (1924). Extraction of the blood in the apparatus was complete after six minutes. Samples of the equilibrated gases were analyzed in the Haldane apparatus. Hematocrit measurements were made with a high speed (6000 r.p.m.) centrifuge.

Each sample of blood was analyzed after equilibration at from two to five temperatures. If the procedure was prolonged, final results were checked by repetition of an early equilibration. It was possible to keep the blood without change at 30° for at least an hour, but after a short time at 35° the oxygen capacity was not restored by equilibration at 15°.

Mixtures of the blood of several fish gave results comparable with the blood of single fish. Blood samples from 23 brook trout, 23 brown trout, and 34 rainbow trout were examined on five separate days for each species. Each species was examined in March, October, November, and December. No fish were used soon after stripping the sperm for breeding, and there was no apparent seasonal change in the blood.

The temperature of the natural spring water at Hackettstown was uniform at about 12°, but in the pools it fell as low as about 6° without noticeably altering the blood. With a good flow of water in the hatchery and stock selected for a number of years conditions are especially favorable for a degree of uniformity that has not been encountered in our experience with several other species of wild fish.

OXYGEN CAPACITY

When equilibrated with air at temperatures below 25° and with pressure of CO_2 less than 2 mm., the hemoglobin is saturated with oxy-

TABLE II

Oxygen capacity

Species	Number of fish	HbO$_2$ ml. O$_2$ per 100 ml. blood	Cell volume ml. O$_2$ per 100 ml. blood
Brook trout *Salvelinus fontinalis*	23	11.7 (11.0–13.9)	40
Brown trout *Trutta trutta*	23	12.2 (11.1–14.4)	35
Rainbow trout *Trutta iridea*	34	13.8 (13.0–15.0)	43

gen. The oxygen combined with hemoglobin was determined by subtracting from the total oxygen in the blood the dissolved oxygen, which was estimated from the solubility coefficients for oxygen dissolved in
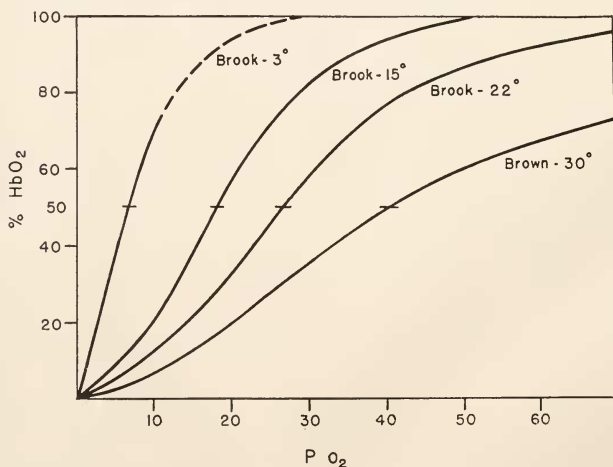

FIG. 2. Oxygen dissociation curves of trout blood at various temperatures.

mammalian blood (Peters and Van Slyke, 1935). The amount of oxygen combined with the saturated hemoglobin is designated as oxygen capacity. Its value is shown for the three species in Table II. Since the samples were frequently mixed from several fish, the variations in oxygen capacity are not the limits of those which may occur among individuals. The oxygen capacities were slightly larger than in the cat-

fish, bowfin, carp, and sucker (Black, 1940), and distinctly larger than
in many of the marine fish examined by Root (1931).

OXYGEN DISSOCIATION CURVES

In our experience the oxygen affinity of fish hemoglobin is not per-
ceptibly diminished by CO_2 unless the tension exceeds 2 mm. The
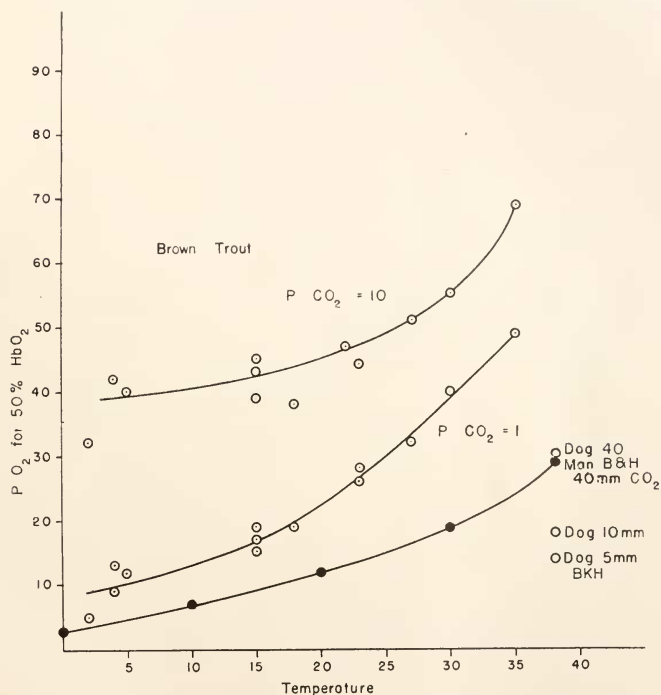

FIG. 3. The effect of temperature upon the pressure of oxygen needed to
half-saturate blood of brown trout at CO_2 tensions 1 and 10 mm. Human blood
(Brown and Hill, 1923) and some points for dogs' blood (Bohr, Hasselbalch, and
Krogh, 1904) are shown for comparison.

oxygen dissociation curves shown in Fig. 2 were made with blood having
tensions of CO_2 less than 2 mm., drawing the best lines through points
from several samples of mixed blood of the brook trout. One sample

of mixed blood from two brown trout was examined over the range of oxygen pressures at 30°. Considering that there are individual varia- tions and that the construction of a number of complete curves for each species at each temperature is not practical, it may be said that these are representative curves, and that one family of similar curves depicts the oxygen affinity of the blood of all three species at various temperatures.

In the gills of trout, loading naturally occurs with tensions of CO_2
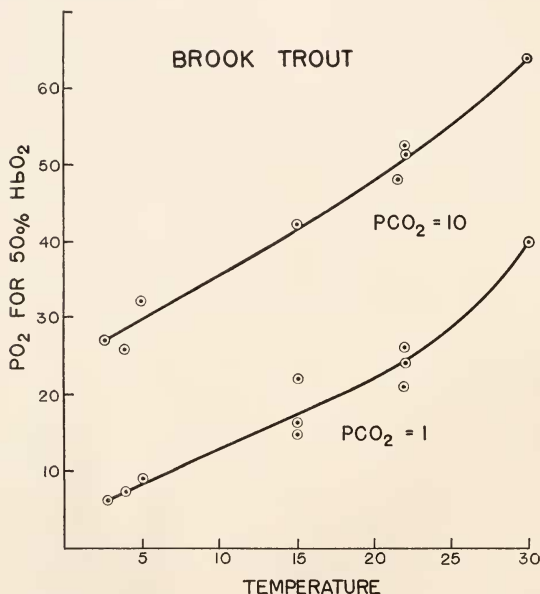

FIG. 4. The effect of temperature upon the pressure of oxygen necessary to half- saturate blood of brook trout at CO_2 tensions 1 and 10 mm.

in the water which are less than 2 mm. (Ferguson and Black, 1940). In the arterial blood of rainbow trout the tension of CO_2 is about as low, and so the curves represent the condition of the blood when loading with oxygen occurs in the gills. At 30° the blood could not be satu- rated with the oxygen pressure of the air, but at 25° the hemoglobin could reach its full saturation.

At temperatures above 15° it is easy to see that the curves are S-shaped. At lower temperatures the deviation is too small to be dem-

onstrated with certainty. At any temperature the curve is sufficiently close to a straight line so that a single determination of the oxygen content and tension of blood between 40 per cent and 60 per cent saturation establishes within 2 mm. the point where the curve cuts half saturation. In this manner it was possible by determining the PO_2 for half saturation of the hemoglobin in one lot of blood at a number of tempera-
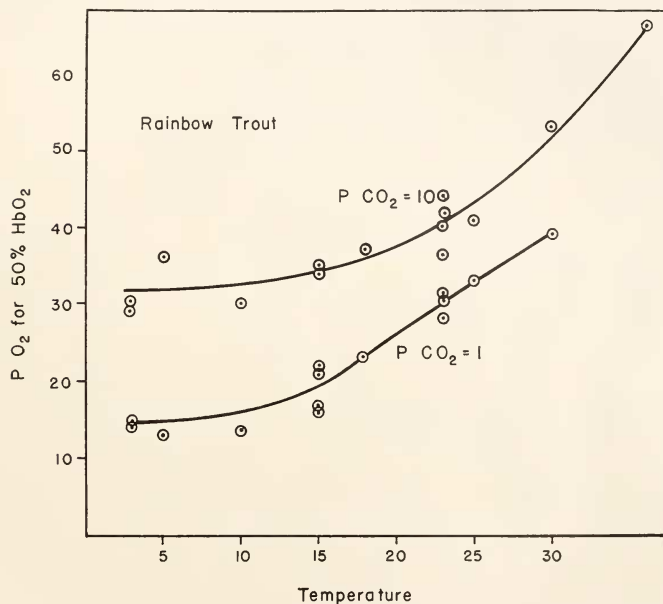

Fig. 5. The effect of temperature upon the pressure of oxygen necessary to half-saturate blood of rainbow trout at CO_2 tensions 1 and 10 mm.

tures, to estimate the effect of temperature upon oxygen affinity before the blood deteriorated.

The effect of temperature upon half saturation of hemoglobin in brown trout blood is shown in Fig. 3, the upper curve at 10 mm. CO_2 tension and the next lower at 1 mm. CO_2. For comparison with human blood the lower curve is drawn from the data of Brown and Hill (1923), and points for dog's blood at 37° (Bohr, Hasselbalch, and Krogh, 1904) show how CO_2 affects its affinity for oxygen. Data for the effect of

temperature upon blood of brook trout and rainbow trout are shown in Figs. 4 and 5.

When the curves for the three species are superimposed in Fig. 6, it is apparent that without CO_2 there is no difference between the blood of brook and brown trout, but the hemoglobin of rainbow trout requires somewhat greater pressure of oxygen to secure half saturation, particularly at lower temperatures. In the absence of CO_2, the bloods of the
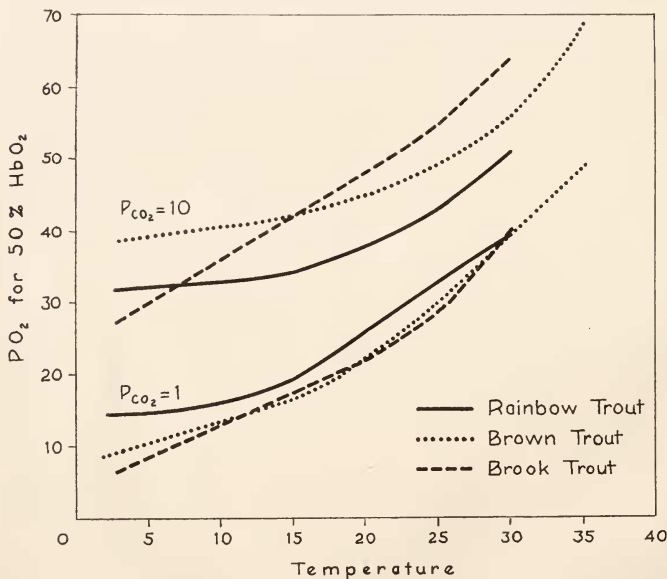

FIG. 6. Comparison of effects of temperature upon oxygen affinity in blood of brown, brook, and rainbow trout.

three trout are quite similar, but with 10 mm. CO_2 tension significant differences are apparent.

In the blood of several fish (Root, 1931; Willmer, 1934; Black, 1940), typical oxygen dissociation curves obtained in the absence of CO_2 are shifted to the right by CO_2. The shape of the curve may be altered as well as its position (Root, 1931), and one change in particular appears in the failure of the hemoglobin to become saturated with oxygen even at tensions of 150 mm. As a consequence of this situation the

tension of O_2 required for half saturation cannot serve as in the absence of CO_2 to define the whole dissociation curve.

Up to about 65 per cent saturation, however, oxygen dissociation curves in the presence of 10 mm. CO_2 rise approximately straight from the origin. If the oxygen tension for a single degree of saturation between 40 and 60 per cent is determined, that point may then be used to designate the curve as far as the 65 per cent level without appreciable error, and the tension at half saturation locates part of the curve as well as the important physiological condition during unloading of oxygen.

The effect of 10 mm. tension of CO_2 upon the oxygen tension needed for half saturation is shown by the upper curves in Figs. 3, 4, and 5 at each temperature. The distribution of points at any temperature amounted to about 10 mm. in the blood of rainbow and brown trout,
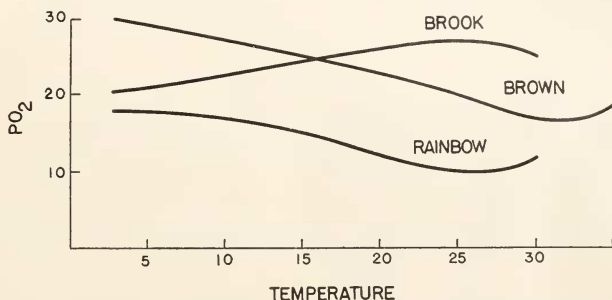

FIG. 7. Increase in PO_2 necessary for half-saturation when PCO_2 is increased from 1 to 10 mm. at different temperatures.

but was less in brook trout. The curves do not miss any point by more than 5 mm., and describe the condition of the blood closely, considering the number of samples of blood examined.

When the curves are superimposed in Fig. 6, it is apparent that at 15° the blood of brook and brown trout are alike with and without CO_2. The effect of CO_2 upon rainbow trout blood is less by nearly 10 mm., which is a greater difference than is shown by individual variations. Over the full range of temperature the curve representing brook trout blood with CO_2 differs from the curve for the other two species in position and slope.

If the addition of CO_2 to the blood occurred in the tissues, the diminished affinity of hemoglobin for oxygen would facilitate the diffusion of oxygen from the blood into the tissues. The situation resembles that

produced by the Bohr effect in favoring unloading of oxygen from human blood, but in the blood of many fish the influence of CO_2 is much greater. In Fig. 7 there is shown the influence which 10 mm. CO_2 would have in raising the oxygen tension at half saturation and at various temperatures. At 5° the addition of 10 mm. CO_2 requires a rise in oxygen tension for half saturation of 17 mm. in rainbow trout, 20 mm. in brook trout, and 30 mm. in brown trout. The oxygen tension is, however, three times increased by CO_2 in brook trout and only doubled in rainbow trout. The practical bearing of specific differences of this size upon the unloading tensions of oxygen would be important in respiratory transport. It is indicated that the blood of the three species suits its use under different conditions, particularly at temperatures away from 15°, but during unloading in the tissues rather than during loading in the gills.

PROPERTIES OF THE ERYTHROCYTES RELATED TO THE CO_2 EFFECT

Effect of Hemolysis

It is an easy custom to attribute differences among different specific types of blood to specific properties of the hemoglobin involved. But the pure hemoglobin of fish is not known, and there are some striking illustrations that in fish blood the properties of hemoglobin depend upon its condition within the erythrocyte. The effects of CO_2 upon O_2 affinity largely disappear from the blood of the carp and sucker when the blood is hemolyzed (Black and Irving, 1938). The CO_2 effect likewise depends upon the integrity of the erythrocytes in the blood of the tautog and toadfish. On the other hand, the hemolyzed blood of the sea robin still retains part of its sensitivity to CO_2 (Root, Irving, and Black, 1939). The blood of the Atlantic salmon (Irving, 1939) is still sensitive to CO_2 after hemolysis, and so there are instances for the dependence of CO_2 sensitivity upon the erythrocytes in some species and independence in other species.

Samples of normal trout blood and of blood hemolyzed with saponin were equilibrated with 150 mm. pressure of oxygen and several pressures of CO_2 up to about 80 mm., according to the procedure outlined earlier for determining the limit of the CO_2 effect. The CO_2 effect in hemolyzed blood proved to be only slightly less than in whole blood. The limit of the CO_2 effect was about 50 per cent reduction of the hemoglobin in whole blood and about 40 per cent reduction in hemolyzed blood. Evidently the hemoglobin of trout blood, like that of salmon and sea robins, is sensitive to CO_2 whether in the erythrocytes or in hemolyzed blood.

Changes in Cell Volume

We have observed that the erythrocytes of a number of fish swell to a remarkable extent when the CO_2 tension is increased. The erythrocytes of suckers (Black, 1940), tautog, sea robins, and toadfish (unpublished observations), and Atlantic salmon (Irving, 1939), and rainbow trout (Ferguson and Black, 1940) swell considerably, while the cells of carp (Black, 1940) swell very little with CO_2. In the rather small number of species examined swelling is great in those in which the CO_2 effect is large, and small where the CO_2 effect is small.

Comparing the erythrocyte volume of the blood of the trout when the blood was equilibrated with air, with the erythrocyte volume when the same blood was equilibrated with 10 mm. CO_2 and about half saturated with oxygen showed that CO_2 caused swelling in every case. These volume changes are shown in Table III as the increase in per-

Table III

Increase in volume of erythrocytes produced by 10 mm. CO_2

Species	Number	Range of swelling	Average of swelling
		per cent	*per cent*
Brown trout	14	1–21	10
Trutta trutta			
Rainbow trout	25	4–27	15
Trutta iridea			
Brook trout	10	1–24	9
Salvelinus fontinalis			

centage over the original volume. Removal of the CO_2 reduced the volume of the erythrocytes again. The swelling of fish erythrocytes with CO_2 is more variable than would be expected if CO_2 tension is the only variable factor which determines volume changes, and erythrocyte volume is certainly labile toward factors or conditions other than CO_2 tension alone.

CO_2 Dissociation Curves

The data from the determinations of CO_2 effects were plotted logarithmically as content against tension of CO_2. In all of the blood samples considered the hemoglobin was about half saturated. The points were uniformly distributed, and the regularity justified the construction of the average curves shown in Fig. 8. The points show the mean position of the results obtained with each species, and indicate that there was no difference between the average blood of brook and brown trout.

Blood of rainbow trout has, however, consistently a somewhat smaller CO_2-combining power than the blood of the others. Judging from the slope of the curves, the buffering of the blood of the three species is similar at CO_2 tensions greater than 10 mm. Up to 10 mm. the buffering of brook and brown trout somewhat exceeds that of rainbow trout, although the concentration of hemoglobin in the latter is usually larger than in the other two species.

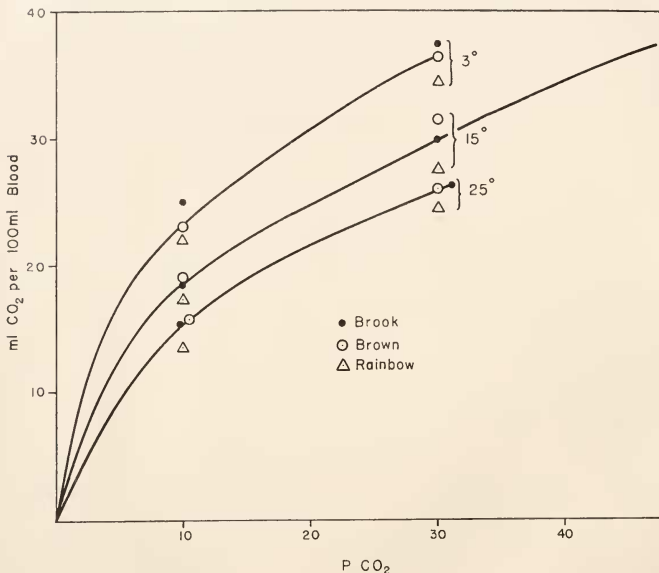The amount of CO_2 combined with blood increases with diminishing

FIG. 8. CO_2 dissociation curves of trout blood at 3°, 15°, and 25°.

temperature more rapidly than does the solubility of CO_2 alone. If the CO_2 added to the blood increases the anion concentration, base must be removed by CO_2 from the protein buffers, which, as weak acids, must then undergo a decline in strength in comparison with carbonic acid. The amount of base lost by proteins and gained by CO_2 is equivalent to the change from 26.0 to 36.0 volumes per cent of combined CO_2 between 25° and 3° or 4.5 millimols per liter. Along with this decline in base-binding power of the protein, the affinity of the hemoglobin for oxygen increases as the temperature declines.

Increasing the temperature diminishes the affinity of the hemoglobin for oxygen and increases the ability of the hemoglobin to bind base. The affinity of hemoglobin for oxygen may also be diminished by acidification, but with an opposite effect upon ability to bind base. From these contrasting relations between the effect of temperature and acidity upon oxygen affinity it appears likely that change of temperature does not alter the affinity of hemoglobin for oxygen by affecting its ionization.

DISCUSSION

The affinity for oxygen of the blood of the three species of trout is scarcely distinguishable at 15° and in the absence of CO_2. In the arterial blood of trout the tension of CO_2 is probably only one or two millimeters (Ferguson and Black, 1940), and complete oxygenation in efficient gills would be equally secured for all three species by atmospheric tensions of oxygen in water cooler than 25°. At 20° half of the atmospheric tension of oxygen would suffice to saturate the blood, and it seems likely that under common natural conditions of temperature and oxygen supply the blood of all three species would be sufficiently and about equally oxygenated.

But when the effects of CO_2 and changing temperature are considered, differences appear which distinguish each species. At 5° the normal tension of CO_2, which is about 10 mm. in venous blood, would yield, at half saturation, as unloading pressure, in brook trout 29 mm., in rainbow trout 32 mm., and in brown trout 39 mm. At 25°, the pressures would be 54 mm. in brook trout, 47 mm. in brown trout, and 39 in rainbow trout. The condition of half saturation and with 10 mm. tension of CO_2 represents the situation in the blood when O_2 is passing into the tissues. At low temperature the tension of O_2 available for unloading would be greatest in brown trout, while the blood of brook trout would offer greater unloading tension at higher temperature. The characteristics of unloading are distinct for each species at all temperatures except at 15°, and the change in unloading conditions with temperature is likewise distinct in the blood of each species. The natural consequence would afford to the brown .trout an unloading tension greater than that of the other three at low temperatures. At high temperature the brook trout would have the advantage of greater unloading tension.

The specific differences which have been shown might be attributed to the possession of hemoglobin of a different type by each species, but on this point there is no evidence. Another view could regard the differences as based upon the influence of the milieu upon the oxygen affinity of a type of hemoglobin common to the three species. Hemoglobin is extremely sensitive to the acidity and salts in which it exists, and in

natural conditions the slope of the CO_2 curve in trout blood is very steep. The changes in acidity within the erythrocyte may be quite rapid, and that the osmotic changes are apparently quite large is indicated by the considerable swelling of erythrocytes produced by a 10 mm. increase of CO_2 tension. We have not seen any specific difference in the erythrocytes which would affect oxygen affinity, but the differences which we have shown are small, and our observations upon the lability of the erythrocytes are gross. The influence of temperature is undoubtedly exerted directly upon the hemoglobin, but temperature probably influences the properties of the erythrocytes as well, and so indirectly affects the hemoglobin by altering its milieu. The lability of the milieu and the sensitivity of hemoglobin are such that the respiratory functions of quite similar hemoglobins might be greatly modified by the milieu.

SUMMARY

Various species of fish possess blood with different characteristic ability for combining with oxygen. These differences appear to fit the blood of each species for the transport of oxygen under special conditions.

In three closely related species of trout the characteristics of oxygen combination are similar at the low CO_2 tension characteristic of arterial blood. The effect of rising temperature upon the combination of oxygen with the blood *in vitro* of *Salvelinus fontinalis, Trutta trutta,* and *Trutta iridea* is to diminish the oxygen affinity. At 15° their blood is half saturated at 17, 17, and 18 mm. tension of oxygen respectively, and changing temperature increases the oxygen tension required for half saturation about 1 mm. per degree. This situation prevails when the tension of CO_2 is about 1 mm., and only at lower temperature does the blood of rainbow trout become distinguishable from the other two in requiring slightly greater oxygen tension for half saturation.

CO_2 greatly decreases the affinity of the hemoglobin for oxygen. The limit of the effect of CO_2 is reached at about 60 mm., and at that tension at 15° the hemoglobin is only half saturated. Raising the temperature diminishes the degree of oxygen saturation in the presence of CO_2.

When the CO_2 tension is 10 mm., half saturation with oxygen requires about twice the tension of oxygen needed in the absence of CO_2. The curves representing change in oxygen affinity with temperature when the CO_2 tension is 10 mm. are different in position or slope for each of the three species. The differences are large enough to fit the blood of each species for oxygen transport under different conditions.

The erythrocytes of trout blood may swell 25 per cent when the CO_2 tension is increased from one to 10 mm. The swelling is observed in the blood of several species of fish having hemoglobin which is sensitive to CO_2. The CO_2 dissociation curves of the three trout are essentially alike and vary in the same manner with temperature.

The difference observed in the blood of these three species would apparently provide different conditions for unloading oxygen in the tissues, and the change of unloading conditions with temperature is peculiar to each species of trout. Only at temperatures above 20° would aeration at the gills normally be restricted.

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